PATENT

ATTORNEY DOCKET NO.: DIVER1220-2

B. Sisson

Art Unit:

Examiner:

1655

Applicant: Application No.:

Jay M. Short 09/214,645

Filed:

September 27, 1999

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IN THE CLAIMS:

Claim 3 has been canceled.

Claims 1 and 2 have been rewritten to read as follows:

(Twice amended) A method for producing a mutant polynucleotide encoding a
polypeptide having a biological activity or a desired property comprising:
blocking or interrupting a polynucleotide synthesis or amplification process by

blocking or interrupting a polynucleotide synthesis or amplification process by contacting a polynucleotide with one or more agents that block or interrupt synthesis or amplification of the polynucleotide wherein the agent is selected from UV light, one or more DNA adducts, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerase, cold or heat, chain terminators, polymerase inhibitors and poisons, and subjecting said polynucleotides to an amplification procedure to provide a mutant polynucleotide.



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- 2. (Amended) A method for producing a mutagenized polynucleotide encoding a polypeptide having a desired property, said method comprising:
 - (a) blocking or interrupting a polynucleotide synthesis or amplification process with at least one member selected from UV light, one or more DNA adducts, DNA intercalating agents, chain terminators, and/or polymerase inhibitors or poisons, wherein said member blocks or interrupts polynucleotide synthesis or amplification so as to provide a plurality of single or double-stranded polynucleotides;
 - (b) denaturing the plurality of single or double stranded polynucleotides to produce a mixture of single-stranded polynucleotides;
 - (c) incubating a plurality of said single stranded polynucleotides with a polymerase under conditions which result in annealing of said single-stranded polynucleotides at regions of homology between the single-stranded polynucleotides and under conditions which promote synthesis of mutagenized polynucleotides, and;
 - (d) expressing at least one polypeptide from said mutagenized polynucleotides; wherein the polypeptide possesses a desired characteristic.

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The following new claims 11-15 have been added:

11. (New) The method of claim 2, wherein said DNA adduct is a member selected from: UV light; (+)-CC-1065; (+)-CC-1065-(N3-Adenine); a N-acetylated or deacetylated 4'-fluro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis; trivalent chromium; a trivalent chromium salt; a polycyclic aromatic hydrocarbon ("PAH") DNA adduct capable of inhibiting DNA replication; 7-bromomethyl-benz-I-anthracene ("BMA"); tris(2,3-dibromopropyl)phosphate ("Tris-BP"); 1,2-dibromo-3-chloropropane ("DBCP"); 2-bromoacrolein (2BA); benzo-I-pyrene-7,8-dihydrodiol-9-10-epoxide ("BPDE"); a platinum(II)halogen salt; N-hydroxy-2-amino-3-methylimidazo(4,5-f)-quinoline; N-hydroxy-2-amino-1-methyl-6-phenylimidazo-(4,5-f)-pyridine, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerases, chain terminators, and polymerase inhibitors or poisons.--

12. (New) The method of claim 2, wherein said DNA adduct is a member selected from: UV light; (+)-CC-1065; (+)-CC-1065-(N3-Adenine); a N-acetylated or deacetylated 4'-fluro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis; trivalent chromium; a trivalent chromium salt; a polycyclic aromatic hydrocarbon ("PAH") DNA adduct capable of inhibiting DNA replication; 7-bromomethyl-benz-I-anthracene ("BMA"); tris(2,3-dibromopropyl)phosphate ("Tris-BP"); 1,2-dibromo-3-chloropropane ("DBCP"); 2-bromoacrolein (2BA); benzo-I-pyrene-7,8-dihydrodiol-9-10-epoxide ("BPDE"); a platinum(II)halogen salt; N-hydroxy-2-amino-3-methylimidazo(4,5-f)-quinoline; N-hydroxy-2-amino-1-methyl-6-phenylimidazo-(4,5-f)-pyridine, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerases, and polymerase inhibitors or poisons.--

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13. (New) The method of claim 2, wherein said DNA adduct is a member selected from UV light; (+)-CC-1065, and (+)-CC-1065-(N3-Adenine).

14. (New) The method claim 2, further comprising releasing and/or removing the DNA adduct prior to (b).

15. (New) The method of claim 2, wherein the DNA adduct is released and/or removed by heating a solution comprising the polynucleotides prior to (b).

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